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Upregulation of Epstein-Barr Virus-Encoded Latent Membrane Protein by Human Herpesvirus 6 Superinfection of EBV-Carrying Burkitt Lymphoma Cells

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The effect of HHV-6 strain A infection on the expression of Epstein-Barr virus- (EBV-) encoded growth transformation-associated genes in two EBV-positive Burkitt lymphoma cell lines, Akata and P3HR-3, was investigated. The results indicate that HHV-6A upregulates the expression of the latent membrane protein LMP-1 in both cell lines. Expression of EBNA-2 was also upregulated in Akata cells following HHV-6A infection. Transfection of reporter constructs carrying the LMP-1 regulatory sequences (LRS; –634/+40) or its 5′ deleted derivatives in Akata and in a T-lymphoblastoid cell line, J-Jhan, confirmed the presence of positive and negative regulatory elements responsive to HHV-6A infection in LMP-1 regulatory sequence (LRS). The majority of LRS constructs were under the influence of dominant negative factors. HHV-6A was able to override the effect of such factors acting on reporter plasmids containing the –634/–54, –324/–54, –214/–54, and –106/–54 parts of LRS. The plasmid that carried only the –54/+40 LRS region was constitutively active in both Akata and J-Jhan cells; in Akata, its activity was influenced by HHV-6A. The finding that HHV-6A infection may activate LMP-1 and EBNA-2 expression, which is essential for the immortalization of B-lymphocytes by EBV, shows a novel aspect of the interaction between these two herpesviruses. ***J. Med. Virol. 55:219–226, 1998.***

KEY WORDS: HHV-6; EBV; LRS; latency; LMP-1

INTRODUCTION

Lymphoblastoid cell lines (LCLs), established in vitro after Epstein-Barr virus (EBV) infection of B-lymphocytes, carry multiple episomal copies of the viral genome and express a set of nine EBV-encoded products as part of the viral program of latent infection [Liebowitz and Kieff, 1993]. These include six nuclear proteins, EBNA 1–6, and three membrane proteins, LMP-1, 2A, and 2B. Phenotypically, LCLs display a number of B-cell surface activation markers (i.e., CD23, CD30, CD39) and the adhesion molecules LFA-1, ICAM-1, and LFA-3. Another form of EBV latent infection is observed in freshly established cell lines from Burkitt's lymphoma (BL), where the expression of viral latent proteins is restricted to EBNA-1. In contrast to LCLs, BL cells have phenotypic traits akin to the one shown by the CD10/CD77-positive lymphocytes populating the B-cell areas of follicle germinal centers. Such BL lines do not display surface activation markers and are termed group I BLs [Rowe et al., 1987; Gregory et al., 1988].

After prolonged passage in vitro, group I BLs shift to an LCL-like phenotype, together with a change in viral gene expression toward the full range of latent proteins (group II/III BLs) [Gregory, 1992]. An intermediate type of viral latency with the expression of EBNA-1, LMP-1, 2A, and 2B occurs in different cell lineages other than the B-lymphocyte, as seen in nasopharyn-

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geal carcinoma (NPC) [Fåhraeus et al., 1988; Young et al., 1988], peripheral T-cell lymphoma (PTL) [Chen et al., 1993], and Hodgkin's disease (HD) [Deacon et al., 1993]. The regulatory mechanisms determining the three different types of latency are only partially understood. Both cellular and viral factors could be involved in latency regulation.

Human herpesvirus 6 (HHV-6) is a ubiquitous lymphotropic herpes virus, etiologically associated with exanthem subitum [Yamanishi et al., 1988]. HHV-6 has a predominantly $CD4^+$ T-lymphocyte tropism [Lusso et al., 1988], although other cell types can be infected in vitro. HHV-6 upregulates CD4 expression in $CD4^+$ T-cells [Lusso et al., 1991] and induces CD4 molecules in $CD8^+/CD4^-$ T-cells and NK cells [Lusso et al., 1991, 1993]. In HIV-infected T-cells, it transactivates the viral LTR, leading to an increase of HIV gene expression [Lusso et al., 1989]. Recently, HHV-6 strain A has been shown to infect also B-cells, provided they are EBV genome-positive, leading to induction of the EBV lytic cycle [Flamand et al., 1993; Cuomo et al., 1995]. Taken together, these observations suggest a possible role for HHV-6A as a cofactor in the pathogenesis of AIDS, by enhancing both the HIV activity and the risk for EBV-associated lymphoproliferative disorders (LPDs).

Three different forms of EBV reactivation pathways are described in response to chemical agents [Rowe et al., 1992]. In the present study, we have investigated whether the EBV reactivation induced by HHV-6A follows any of the previously described pathways. For this purpose, two EBV-positive BL lines, Akata and P3HR-3, were chosen. The results indicate that HHV-6A infection induces the de novo synthesis of LMP-1 and EBNA-2 in Akata and upregulation of LMP-1 in P3HR-3 cells. Using the complete LMP-1 regulatory sequence (LRS) and a series of LRS 5′ deleted constructs linked to the chloramphenicol acetyltransferase (CAT) reporter gene, it was observed that the transactivating signals put in action after HHV-6A infection are able to counteract the activity of negative regulatory factors dominating in the B- and T-cell environment.

MATERIALS AND METHODS

Cell Lines

The immature T-cell lines J-Jhan and HSB-2 [Adams et al., 1968], the EBV-positive BL lines Akata [Takada et al., 1991] and P3HR-3 (a subclone of P3HR-1) [Hinuma and Grace, 1967], the EBV-negative BL lines BL28 and BL41 [Lenoir et al., 1985], and the EBV producer line B95-8 [Miller and Lipman, 1973] were maintained in RPMI 1640, supplemented with 2-mM glutamine, 100-IU/ml penicillin, 100-μg/ml streptomycin, and 10% fetal calf serum (complete medium). Akata expresses only EBNA-1 and retains a stable group I phenotype. P3HR-3 is a group II BL and carries a deletion in the EBNA-2 and EBNA-5 coding regions. P3HR-3 expresses all the other EBNAs and very low levels of LMP-1. Akata cells were periodically tested for EBNA expression by ACIF [Reedman and Klein, 1973] and found 100% positive. Phosphonacetic acid (PAA) treatment was performed adding 0.8-mM PAA (Sigma, St. Louis, MO) in complete medium 7 days before HHV-6 infection.

Virus Preparation and Infection

HHV-6A (GS strain) was propagated in HSB-2 cells as described previously [Ablashi et al., 1988; Lusso et al., 1988]. When 90% of the cells showed the characteristic cytopathic effects of HHV-6A infection (presence of large, refractile cells) [Salahuddin et al., 1986], the cell suspension was harvested, freeze–thawed three times, and the virus concentrated by ultracentrifugation (29,000 g) for 90 min at 4°C. The pellet was carefully suspended in 1/100 volume of RPMI supplemented with 50% FCS and kept at −80°C.

EBV-carrying cell lines were infected with HHV-6A as follows: 5×10^6 cells were incubated with 1 ml of stock virus (titer 10^5 $TCID_{50}$) for 2 hr at 37°C. The cells were washed and resuspended in complete medium. At different time intervals, the cells were harvested, processed for fluorescence microscopy, Western blotting, or transfections. All washing procedures were carried out in phosphate-buffered saline (PBS, pH 7.4).

Immunofluorescence Analysis

Acetone-methanol (1:2 vol/vol) fixed cell smears were prepared for the estimation of fluorescent cells by indirect two-step immunofluorescence (IF) [Lai et al., 1973]. For each experiment, a minimum of 350 cells were counted.

For surface markers, the cells were incubated with monoclonal antibodies to cell surface antigens. Fluorescein-conjugated goat antimouse immunoglobulin G (IgG) was used as secondary antibody (Cappel, Durham, NC). The stained cells were analyzed on a Becton Dickinson FACS.

Western Blotting

The procedure for Western blotting has been fully described elsewhere [Laemmli, 1970; Towbin et al., 1976]. Briefly, after lysis in the sample buffer and sonication, 1.5×10^6 cells were loaded onto discontinuous 7.5% SDS-polyacrylamide gel. The electrophoresed proteins were transferred onto nitrocellulose filters, blocked with milk overnight, and incubated with monoclonal antibodies. Immunoreactive proteins were visualized by chemiluminescence with the ECL detection kit (Amersham, Arlington Heights, IL). Western blot analysis of both EBNA2 and LMP1 was performed on paired filter blots with samples derived from a common source of cell lysates.

Analysis of EBNA-2 Expression by RT-PCR

Total RNA was extracted by acid guanidium-thiocyanate phenol-chloroform method [Chomczynski and Sacchi, 1987]. First strand cDNA was synthesized from 3 μg of total RNA by using Moloney Murine leukemia virus (M-MLV) reverse transcriptase (Gibco BRL, Life Technologies Co., Greithersburg, MD).

Briefly, the extracted RNA was incubated with 20 μl of reaction buffer containing 50-mM Tris-HCl (pH 8.3), 75-mM KCl, 5-mM $MgCl_2$, 10-mM dithiotreitol, 0.5-mM deoxynucleotide triphosphates (dNTPs), 100-pmol random hexamers (GIBCO), 20 U of RNAse inhibitor (Promega), and 200 U of M-MLV for 60 min at 42°C and then for 5 min at 95°C. Polymerase chain reaction (PCR) was carried out in a 50-μl volume containing 10 μl of 1:3 diluted cDNA, 2.5-mM $MgCL_2$, 1 × PCR buffer, 200 μm each dATP, dGTP, dCTP, and dTTP, 1-μM primers, and two units of Amplitaq Gold (Perkin Elmer Co, Norwalk, CT). Primer sequences for EBNA-2 transcript and PCR conditions are described elsewhere [Chen et al., 1995]. For control, β-actin was amplified using the primers: 5′-CGAGCGGGAAATCGTGCGTGACATTAAGGAGA-3′ and 5′-CGTCATACTCCTGCTTGCTGATCCACATCTGC-3′ (Clontech, 5403-1). The amplification was carried out according to the manufacturer's specification. The PCR products were visualized by electrophoresis in 2% agarose gel. The PCR products were blotted onto nylon filters (Hybond N; Amersham) and detected by Southern hybridization using a specific oligonucleotide probe end labeled with (γ–^{32}P)ATP.

Monoclonal Antibodies

The following monoclonal antibodies (MAb) were used: MAb S12, which detects the carboxy-terminal part of LMP-1 [Mann et al., 1985]; MAb PE2, which is directed against EBNA-2 (Dakopatts) [Young et al., 1989]; and MAb R3, directed against the EA-D protein complex encoded by BMRF-1 [Pearson et al., 1983]. MAb 41/38 (ABI, Columbia, MD) detects the HHV-6-encoded phosphoprotein p41 [Cheow and Balachandran, 1991]. MAb MHM23 (anti-CD18) reacts with the common β-chain of the LFA-1 molecule [Hildreth et al., 1983], whereas MHM24 is specific for a member of the α-chain family (CD11α) of the LFA-1 heterodimer [Hildreth et al., 1983]. RR/1 reacts with the adhesion molecule ICAM-1 (CD54) [Patarroyo et al., 1986], and TS2/9 detects LFA-3 (CD58) [Sanchez-Madrid et al., 1982].

Plasmids

The LRS-CAT reporter plasmids include DNA fragments derived from the B95-8 EBV strain genome [Baer et al., 1984], spanning nucleotides +40 to different upstream positions relative to the ED-L1 promoter transcription initiation site (+1). The pgLRS(-634)CAT, and the 5′ deleted derivatives pgLRS(–324)CAT, pgLRS(–214)CAT, pgLRS(–106)CAT, pgLRS(–54)CAT, and pgCAT, are described in detail elsewhere [Fåhraeus et al., 1990]. The EBNA-2 expression plasmid pEΔA6 has been described previously [Ricksten et al., 1987].

Transfection and CAT Assay

Transfection of Akata and J-Jhan cells with LRS CAT constructs and control plasmids was carried out by electroporation. A total of 10^7 cells were resuspended in 0.2-ml PBS with 10-μg plasmid DNA, and electroporated with the Gene Pulser apparatus (Bio Rad) at 250 V and 960 μF. Cotransfections were carried out using 20 μg of LRS-CAT reporter plasmids and 16 μg of EBNA-2 expression plasmid. Cell extracts were prepared 48 hr after transfection and CAT activity was analyzed as described previously [Gorman et al., 1982]. The optimum timing for plasmid electroporation and CAT assay after HHV-6A infection was determined. For this purpose, Akata and J-Jhan cells were mock- or HHV-6A-infected and transfected with the pgLRS(–634)CAT plasmid at 24 hr, 72 hr, and 5 days after the infection. Cells transfected 24 hours post-HHV-6A infection showed maximum CAT activity. Data were quantified by autoradiography and liquid scintillation counting.

TABLE I. Time Course of HHV-6A/p41 Expression After HHV-6 Infection[a]

	% p41 positivity, days after infection[b]				
Cell lines	2	3	9	13	21
Akata	5.1	5.3	1.2	0.12	0
P3HR-3	10.7	9.3	5.4	0.23	0.08

[a]HHV-6/p41 expression was examined by immunofluorescence using the MAb p41/38 (ABI, Columbia, MD). Results are expressed as percent positive cells out of at least 350 cells counted. One representative experiment out of five.
[b]Cells were incubated for 2 hours at 37°C in the presence of the virus as described in Materials and Methods. At the indicated time, cells were harvested, washed, and stained for p41 expression.

RESULTS

HHV-6A Infection of P3HR-3 and Akata

It was shown previously that HHV-6 infection leads to EBV lytic cycle antigens induction in EBV-positive B-cell lines [Cuomo et al., 1995]. In the present study, the replicative activity of HHV-6 and subsequent EBV lytic antigen induction was examined in P3HR-3 and Akata. The kinetics of HHV-6 infection as judged by the expression of the HHV-6-encoded early antigen p41 is shown in Table I. The highest levels of p41-positive cells were obtained 48–72 hr after HHV-6A infection. The decreasing p41 expression, however, can not exclude that HHV-6 could still be present in infected cells. Induction of EBV lytic cycle in P3HR-3 was demonstrated by an increase of EBV-EA-positive cells at 72 hr post-HHV-6 infection (on average from 3.7% cells to 8.2% EA-positive cells in uninfected and HHV-6-infected cells, respectively). Similar induction levels had been reported previously [Cuomo, et al., 1995]. The kinetics of EBV lytic antigens induction in Akata is shown in Figure 1. Western blot analysis confirmed the EBV-EA induction at 72 hours and its persistence for at least two weeks. In both cell lines, the viability was not affected throughout the infection experiments remaining around 90%.

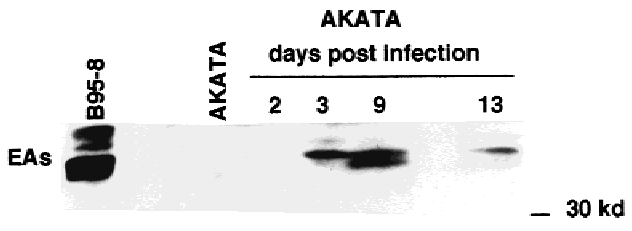

Fig. 1. Immunoblot showing the kinetics of EA-D induction in Akata cells following HHV-6 infection. Replicate cultures of the Akata-BL line were infected with GS strain of HHV-6 and harvested at the indicated time points. EBV producer line B95-8 was used as control. The blot was probed with the monoclonal antibody R3 (diluted 1:50) and proteins were visualized by chemiluminescence.

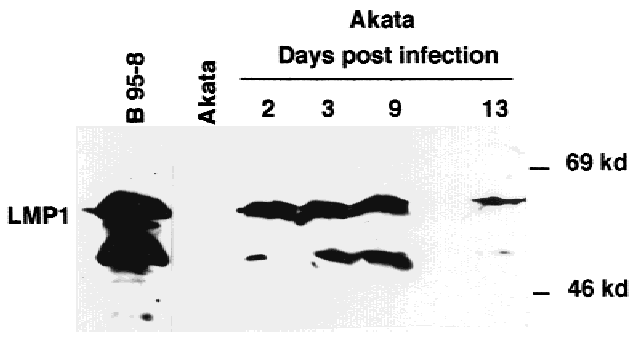

Fig. 2. Immunoblot showing the kinetics of LMP-1 induction after HHV-6 infection in Akata. Proteins derived from the same samples used for EA-D detection were separated by SDS-PAGE and the blot was probed with the monoclonal antibody S12 (diluted 1:1000).

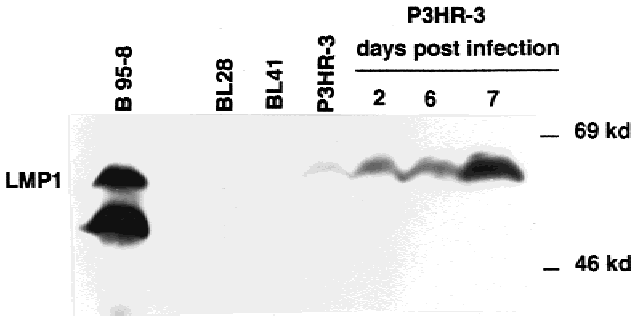

Fig. 3. LMP-1 upregulation in P3HR-3 after HHV-6 infection. The EBV-negative BLs, BL41, and BL28 and the EBV producer cell line B95-8 were used as controls. The blot was probed with the monoclonal antibody S-12.

EBV-LMP-1 Upregulation Following HHV-6 Infection

Akata is a group I BL and does not express LMP-1. As shown in Figure 2, infection with HHV-6A led to LMP-1 protein expression already at 48 hr as detected by Western blot. LMP-1 was still detectable after 13 days post-HHV-6A infection and was absent at 21 days. Both the 63- and the 48-kD forms of LMP-1 were induced in Akata. P3HR-3 BL normally expresses very low levels of LMP-1. After HHV-6A infection, LMP-1 expression was significantly upregulated (Fig. 3). The 48-kD truncated LMP is observed only in cells infected lytically [Rowe et al., 1992]. In five independent experiments, the 48-kD form of LMP-1 was not induced in P3HR-3.

LMP-1 can mediate a number of phenotypical changes in a B-cell environment, including upregulation of cell adhesion molecules [Wang et al., 1987, 1988]. We therefore investigated whether such changes were induced after HHV-6 infection in Akata. FACS analysis did not reveal any quantitative difference in the expression of the adhesion molecules LFA-1α, LFA-1β, LFA-3, and ICAM-1 between mock- and 72-hour HHV-6 infected cells (data not shown).

EBNA-2 Induction in HHV-6A-Infected Akata

EBNA-2 expression was analyzed in Akata at several time points after HHV-6 infection, over a period of 13 days, by Western blotting and RT-PCR. In P3HR-3, EBNA-2 gene is deleted. Western blot analysis of EBNA-2 expression showed a detectable level of the protein only at the ninth day postinfection (Fig. 4A). However, gene transcripts were detected at 48 hours by RT-PCR (Fig. 4B). EBNA-2 transcripts were detected using a primer combination selected for the ability to detect C or W promoter-derived products spliced into the BYRF-1 exon [Chen et al., 1995]. The specificity of PCR products was confirmed by hybridization of the amplified fragments to a specific oligonucleotide probe (not shown).

Mapping of LMP1 Promoter Regions Involved in Its Upregulation by HHV-6A

We next attempted to map the regulatory regions on the LMP-1 promoter, which interact with the deregulation signal generated during HHV-6A infection. To this purpose, a series of reporter constructs carrying either the intact promoter of LMP-1 (from position −634 to +40 with respect to the transcription start site) or the 5′ deleted fragments of LRS upstream of the CAT gene [Fåhraeus et al., 1990] was assayed by transient transfection in Akata and in J-Jhan after HHV-6A infection.

Electroporation was undertaken 24 hr after HHV-6A infection. The basal CAT activities obtained with pg-CAT, a control plasmid without promoter, were similar in mock- and HHV-6-infected cells (1.2%) and were subtracted from the values obtained with the LRS-CAT constructs. All plasmids had a very low activity in mock-infected cells, with the exception of pgLRS(−54)CAT. Following HHV-6A infection, there was a 27-fold increase of CAT activity in Akata cells transfected with pgLRS(−106) (Fig. 5A). Lower levels of CAT activity, corresponding to a 4- to 13-fold activation compared to the mock-infected cells, were observed after transfection of all other reporter constructs (Fig. 5A). LMP-1 is transcriptionally regulated by EBNA-2 [Abbot et al., 1990], although few exceptions have been observed [Fåhraeus et al., 1988; Young et al., 1988]. We therefore sought to compare the pattern of activation of LRS following HHV-6 infection with that obtained after transfection of an EBNA-2 expression construct in the same cell. A similar pattern of LRS induction, although at lower levels of activation compared to those

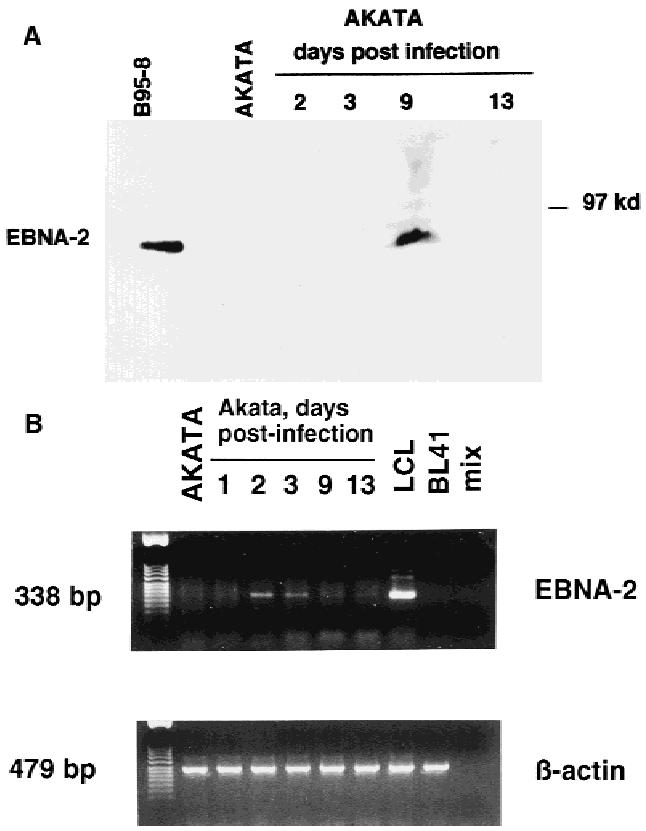

Fig. 4. EBNA-2 expression in HHV-6-infected Akata by **(A)** Western blot and **(B)** RT-PCR. Samples from the same infection experiment were processed for RNA and protein analysis. MAb PE2 was used to detect EBNA-2 protein at the dilution 1:50. As control, RNA was extracted from an EBNA2+ lymphoblastoid cell line (RGN-1) and from the EBV-negative BL cell line BL41. Amplification of β-actin gene served as RNA quality control. 50-bp step ladder (Sigma) was used as PCR marker.

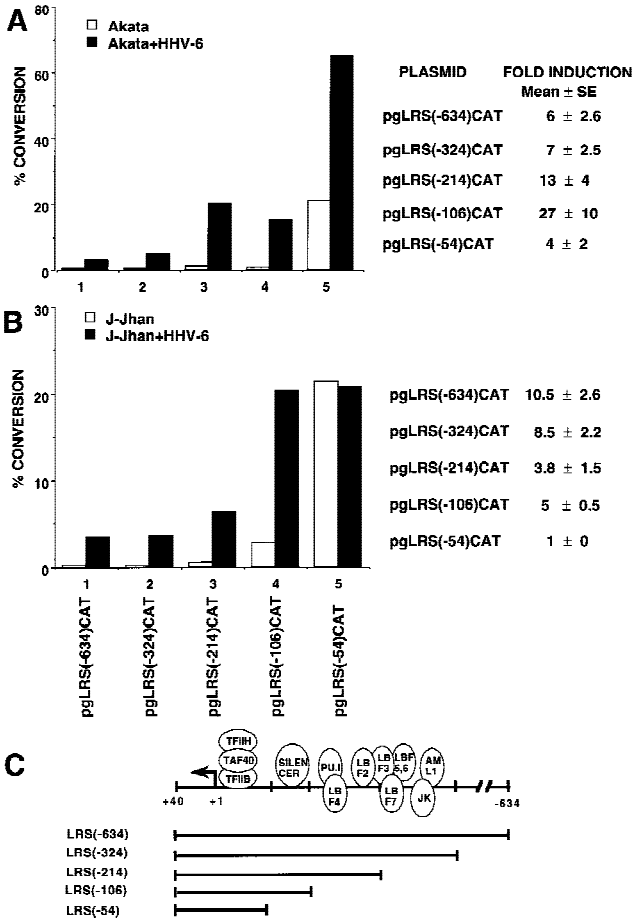

Fig. 5. Activity of LRS-CAT constructs in mock and HHV-6 infected Akata **(A)** and J-Jhan **(B).** The percentage of conversion is calculated according to the formula: [cpm acetylated/ cpm (acetylated + not acetylated)] × 100. The basal CAT activities obtained by pgCAT were subtracted from the values obtained with the LRS-CAT constructs. One representative experiment out of five and fold induction values (mean ± SE) are shown in A for Akata and in B for J-Jhan. The fold induction is calculated as the ratio between the percentage of conversion obtained after HHV-6A infection and that obtained in mock-infected cells. **C:** Schematic representation of LRS-CAT constructs and regulatory elements (adapted from Kieff [1996]).

obtained after HHV-6A infection, was found following EBNA-2 transfection (data not shown). In J-Jhan cells, HHV-6A infection produced a 4- to 10-fold activation of all LRS reporter plasmids with the exception of pgLRS(−54)CAT, which showed the same activity levels as those in the mock-infected cells (Fig. 5B). A schematic representation of LRS-CAT construct and the regulatory regions present on the LRS sequences are shown in Figure 5C (adapted from Kieff [1996]).

Activation of LRS in Presence of Phosphonacetic Acid

In order to define whether LMP-1 induction might be due to early or late gene products of HHV-6, the LRS-CAT activation experiments were repeated in the presence of phosphonacetic acid (PAA, 0.8 mM), which is known to inhibit viral DNA polymerase. On average, 70% to 100% inhibition of LRS activation following HHV-6 infection was achieved in the presence of PAA, depending on the construct tested (Fig. 6). CAT activities were unaltered in cells treated with only PAA. Control experiments using B95-8 cells showed a full inhibition of EBV VCA expression.

DISCUSSION

It has been shown that immunocompromised patients are prone to develop EBV-associated B-cell lymphomas [Herbst et al., 1990]. This immunosuppression, either acquired as in AIDS or therapeutic as in transplantation, seems to interfere with normal immunological responses that usually prevent the EBV-induced clonal proliferation from the infected cells. Lytic reactivation of EBV, in vivo, is also of importance not only for the serious complications associated with recurrent active infections but also for expansion of the resident pool of EBV genome-positive cells through the recruitment of newly infected cells, which would in turn increase the risk for viral-induced proliferation in immunocompromised patients. In this context, HHV-6A, which is an opportunistic agent in several immune disorders, may play an important role by inducing EBV

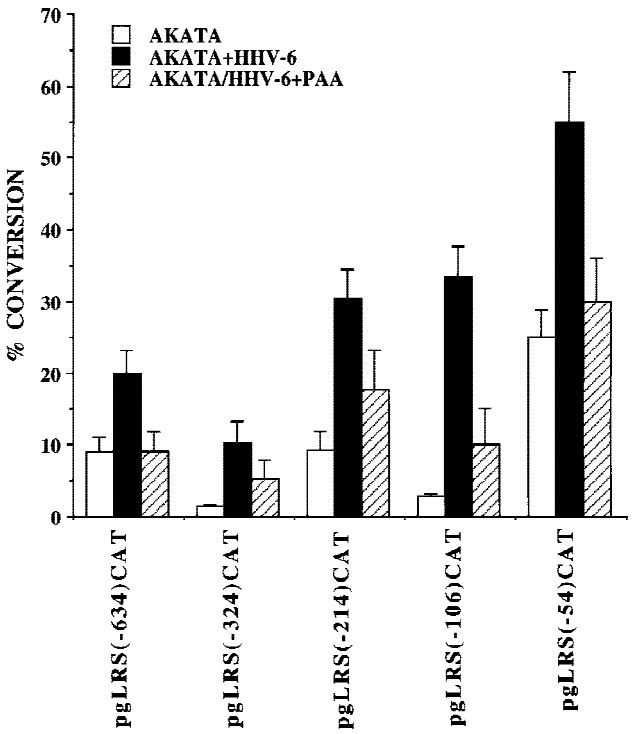

Fig. 6. Activity of LRS-CAT constructs in mock- and HHV-6-infected Akata in the presence of PAA. PAA (0.8 mM) was added in the medium 7 days before HHV-6 infection. See legend of Figure 5. Mean + SE of three different experiments.

reactivation in EBV-carrying cells [Flamand et al., 1993; Cuomo et al., 1995]. In this article, a novel aspect of HHV-6A and EBV interaction is highlighted, showing that HHV-6A not only induces EBV-encoded proteins associated with the lytic cycle but also upregulates expression of EBV-encoded growth-transformation-associated proteins, namely LMP1 and EBNA-2. As indicated by the studies on the LRS, HHV-6A overrides the effect of cell-dependent negative factors influencing the LMP1 expression in both the B- and T-cell environment.

The studies on EBV latency in vivo on peripheral blood B-lymphocytes showed a heterogeneous pattern of latent protein expression, which cannot be related with that of LCLs or BL lines at different stages of differentiation. LMP-1 gene expression was rarely detected and EBNA-2 transcription was not documented [Tierny et al., 1994; Chen et al., 1995; Decker et al., 1996; Gonnella et al., 1997]. It is from the population of EBV-carrying resident cells that EBV-related LPDs may arise in the immunocompromised host. An important missing point about the pathogenesis of these disorders concerns the nature of stimuli and type of the molecular mechanisms that are needed for the proliferation of the cells latently infected by EBV. As suggested by the in vitro studies using EBV recombinants, a number of latent genes seem to promote cell survival and proliferation [Cohen et al., 1991; Tomkinson et al., 1992; Kaye et al., 1993]. To this effect, EBNA-2 and LMP-1 play a pivotal role and cannot be dispensed with. The present report shows that these genes may indeed be activated in the course of HHV-6A infection in BL cells that express a limited set of EBV-encoded genes.

An important question is whether the EBV latent gene activation is associated to an HHV-6A driven shift of the cellular phenotype or whether it is directly mediated by HHV-6A gene functions. The EBV latent gene promoters switch according to the phenotype of the host cell as observed during long-term culture of group I BL [Altiok et al., 1992]. Moreover, LMP-1 and EBNA-2, when transfected in EBV-negative cell lines, themselves have transactivating potential leading to upregulation of cellular genes coding for cell activation markers and adhesion molecules [Wang et al., 1987, 1988]. The results obtained by FACS analysis did not reveal any quantitative differences in the expression of the adhesion molecules between mock- and HHV-6A-infected cells. Our findings are in line with earlier observations where LMP1 induction in association with lytic cycle did not alter the phenotype of the cells [Rowe et al., 1992]. Moreover, it was found that EBNA-2 induction at the protein level is delayed with respect to LMP-1 expression, suggesting a pattern of gene activation compatible with that observed in the same cell line after anti-IgG treatment [Rowe et al., 1992].

The studies on LMP1 promoter induction by HHV-6A in B- and T-cells indicate the presence of regulatory elements in LRS responsive to HHV-6A infection. Similar to our previous finding in other cell types [Fåhraeus et al., 1990], the promoter was constitutively active in the context of the −54/+40 region of LRS, but under the influence of dominant negative elements located further upstream. A negative element was located in the −106/−54 region of LRS. The same region is also involved in the transactivation of the LMP-1 promoter by EBNA-2 in B-lymphoid cells [Fåhraeus et al., 1994; Sjöblom et al., 1995]. In these cells, however, regulatory elements located further upstream in the −176/−136 and the −223/−217 regions, respectively, from a quantitative point of view seem to be more important than the −106/+40 elements for the EBNA-2-induced activation of the promoter [Laux et al., 1994a, 1994b; Johannsen et al., 1995]. In Akata cells (but not in J-Jhan cells), elements in the −176/−136 region of LRS may mediate HHV-6A induced activation of the LMP-1 promoter (Fig. 5 and unpublished results). We suggest the following two interpretations of the results. One, HHV-6A infection activates the LMP-1 promoter by overriding the effect of a negative transcription factor(s) interacting with the −106/−54 region of LRS. This can occur through a reduction of the activity or the amount of the negative factor in the cells or by an increase in the activity/amount of positive transcription factors that act on the −106/+40 LRS region. Two, negative elements in the −634/−106 region reduce but do not completely inhibit promoter activity mediated by HHV-6A in infected B- but not T-cells.

It is unclear at present whether transactivation of LMP1 is related to a direct effect of HHV-6A gene or

mediated through cellular genes. The LRS-CAT experiments performed in the presence of PAA, a viral DNA replication inhibitor, suggest that HHV-6-encoded late gene products could be involved in LMP-1 induction. A more detailed study specifically addressing the question of which viral genes are involved in EBV genes transactivation will be required to clarify this issue. In conclusion, this study provides an experimental framework for further studies on the molecular mechanisms underlying the lymphoproliferative events occurring as a consequence of immunosuppression and reactivation of herpes viral infections.

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